IMMUNOHISTOCHEMICAL STAINING FLUORESCENCE PROTOCOL

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TISSUE PREPARATION FOR SECTIONING

1. Label 15mL aluminum liquid N_2 containers (Accurate Chemical Scientific, 1-800-645-6264) containing 4mL of frozen tap H_20.
2. Sacrifice animals and drop the desired tissues in the aluminum container filled with liquid N_2. Keep groups separated. When liquid N_2 has evaporated, cap the container and store at –70°C.
3. To begin sectioning, mount the individual frozen tissue with tissue freezing medium (Scientific Products, 1-800-964-5227) and allow to freeze thoroughly.
4. Routine sections are trimmed at 20 μm and cut for slides at 5 to 8 μm and picked up on a labeled glass slide.

FLUORESCENT STAIN

1. Remove frozen slides from the –70°C freezer and allow to warm to room temperature.
2. To contain the staining solutions, encircle the section including the tissue medium with a hydrophobic slide marker (PAP pen, #00-8888, Zymed Laboratories, Inc., 800-874-4494).
3. Rinse in 1X PBS.
4. Prepare antibody dilution(s): Dilute antibodies in 0.1% BSA/PBS + 10% rat sera (to block non-specific binding sites). Two to three antibodies may be diluted together, as long as there are no species/isotype conflicts.
5. Wipe the backs and edges of the slides dry (avoiding tissue). Apply 200μL of properly diluted (~1-5 μg/mL) antibody/antibodies to slides and incubate in humid chamber for 45 minutes at room temperature. Cover to avoid exposure to light.
6. Rinse in 1X PBS.
7. Wipe the backs and edges of the slides dry (avoiding tissue). Add 200μL of detection reagent – streptavidin or secondary antibodies (streptavidin-Cy3, -Cy5, and α-rat-IgG-Cy3, Jackson ImmunoResearch, 800-367-5296; α-rat-IgG-FITC, Serotec, 800-265-7376). Incubate slides in humid chamber for 45 minutes at room temperature. Cover to avoid exposure to light.
8. Rinse in 1X PBS. Quickly dip in ddH_2O.
9. Wipe the backs and edges of the slides dry (avoiding tissue); allow tissue to air dry briefly. Add a small amount of Fluoromount-G (#0100-01; Southern Biotechnology Associates, Inc.; 205-945-1774) and apply a coverslip.
10. View slides with the Zeiss fluorescent microscope (B3136), using the Intelligent Imaging Innovations Software.